New insights into the Kimberley phytoplankton and their ecology

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Abstract

The Kimberley is a remote region where the eastern edge of the Indian Ocean interacts with the broadening continental shelf of Northern Australia to generate massive tides. During a 2010 research voyage the phytoplankton communities of the region were elucidated from a combination of light microscopy, remote sensing and size fractionated pigment analysis. In strong contrast to previous work from the NW Shelf and the Gulf of Carpentaria ≥ 80% of the phytoplankton at the shelf break (~ 200 m water depth) and further offshore were found to be < 2 μm (picoplankton) and dominated by *Synechococcus*. Streaks of *Trichodesmium* were visible but cell counts suggested they were only the 9th most abundant taxa. Pigment analysis indicated coccolithophorids were consistently ~ 20% of the total phytoplankton biomass across the region of the cruise. Shelf scale blooms of coccolithophorids are periodically reported in the shallow seas of Northern Australia but only small blooms were observed in MODIS (Moderate Resolution Imaging Spectroradiometer) true colour images from the Kimberley region during the voyage. In shallower waters closer to shore the concentration of phytoplankton rose dramatically. There were concomitant changes in community composition including a decline in *Prochlorococcus* and pelagophytes and a rise in the diversity and abundance of medium to large diatoms. This distinctive, near shore, diatom community was spatially heterogeneous and largely composed of species previously reported as rare in northern Australia.

Keywords: ecology, biogeography, picoplankton, pigments.

Introduction

The Kimberley region is a remote and poorly characterised region of Australia that is currently under significant development pressure associated with resource extraction. In the Kimberley the eastern edge of the Indian Ocean interacts with the broadening continental shelf to generate massive tides. These tides generate considerable mixing creating ecological niches that are not found anywhere else in Australia. Like most of the regional ecology, the phytoplankton are virtually unknown. Joseph Banks noted the presence of *Trichodesmium* in the region during Captain Cook's 1768 to 1771 exploration of the region (Beaglehole 1962). A handful of samples were reported on by Wood (1954, 1964a, 1964b) and a single net sample by Hallegraeff & Jeffrey (1984). In this region variations in ocean colour as observed by satellites have been interpreted as massive coccolithophorid blooms (*e.g.* Brown & Yoder 1994) yet there has been no *in situ* validation and no research into the impacts of these blooms on carbon cycling and acidification.

The general biogeography of phytoplankton around Australia was broadly investigated by Wood (1954, 1964a, 1964b) with more regional investigations of phytoplankton in the north and northwest of Australia by Hallegraeff & Jeffrey (1984) and Tranter & Leech (1987). Most studies of Australian phytoplankton biogeography (*e.g.* Wood 1954, Hallegraeff & Jeffrey 1984, Jeffrey & Hallegraeff 1990) have relied heavily upon net samples which produces a bias against smaller cells. Hallegraeff & Jeffrey (1984) reported that < 3% of the chlorophyll *a* was > 15 μm in the Australian tropics and the majority of cells were nanoplankton (nominally 2 to 15 μm). Subsequent research has shown that the majority of east coast phytoplankton can be < 2 μm (Crosbie & Furnas 2001, Sorokin & Sorokin 2009). The realization that so many of the dominant taxa in the Australian tropics are small suggests the need for some reappraisal of the phyto-biogeography in this region.

Small phytoplankton are well known to contribute significantly to phytoplankton biomass in oligotrophic open waters particularly the prokaryotic cyanobacteria *Synechococcus* and prochlorophyte *Prochlorococcus* which are often reported to be the numerically dominant species (Olson *et al.* 1990). These small cells which often lack taxonomically useful morphological features for identification when observed by epifluorescence microscopy or conventional light microscopy can, fortunately, be estimated from phytoplankton pigments. In this research pigments that are associated exclusively with one taxonomic group and those with strong associations with a particular taxon are used to investigate the relative abundances of the major taxonomic groups (after Jeffrey *et al.* 1997). For example, pigments such as divinyl chlorophyll *a* (DVchl*a*) are found only in *Prochlorococcus*, zeaxanthin found largely in cyanobacteria such as *Synechococcus*, peridinin (dinoflagellates), alloxanthin (cryptophytes), prasinoxanthin (prasinophytes), 19'-hexanoyloxyfucoxanthin (haptophytes), fucoxanthin

(diatoms), and 19'-butanoyloxyfucoxanthin (pelagophytes).

This paper examines the spatial patterns of the phytoplankton in the Kimberley region as observed on a 3 week research voyage during April to May 2010. A combination of light microscopy, high performance liquid chromatography (HPLC) of phytoplankton pigments and remotely sensed ocean colour were used to investigate the spatial and temporal distributions of phytoplankton in the region. The voyage was planned to occur during the development of the seasonal bloom that peaks in early winter (Fig. 1A). The voyage consisted of a series of onshore – offshore transects from near the coast to > 1000 m water depth. On two occasions samples were collected inside King Sound, once on the spring tide and once on the neap tide. The tides in this region of Australia are amongst the world's largest reaching > 10m. These tides, along with some strong internal waves, provide considerable vertical mixing forces that are proposed to be important in the phytoplankton ecology of the region (Tranter & Leech 1987).

Methods

Data sources and description of data analysis

The research voyage commenced on April 14, 2010 and consisted of 195 vertical profiles of the water column along 5 onshore to offshore transects (Fig. 1B). The 195 profiles were obtained at water depths of: ~ 50, 100, 200, 300, 500, 750, 1000, and 2000 m on each transect. A Seabird SBE 911 instrument (CTD) was used to measure conductivity (converted to practical salinity units), pressure (converted to depth (m)) and temperature (°C). Most vertical profiles also contained photosynthetically active radiation (PAR, 400 to 700 nm, Biospherical Instruments QCP-2300), fluorescence (Chelsea Instruments Aquatracka™ fluorometer), % transmission (Wetlabs C-Star™), dissolved oxygen (Anderra 3975 series optode) and nitrate (Satlanic ISUS sensor) concentrations.

Statistical analysis of the data was undertaken in SigmaPlot version 11. Data sometimes failed tests for homogeneity of variance or for normality even when transformed. When this was the case a nonparametric test was used. The exception was 3 way ANOVA, for which the software did not have a nonparametic equivalent, and it was necessary to rely upon the robustness of ANOVA to deviations from normality and heterogeneity (Sokal & Rohlf 1995).

Remotely sensed observations

The seasonal distribution of phytoplankton were assessed using the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) which operated from September 1997 and collected ocean colour data in the visible and far red region of the spectrum (412, 443, 490, 510, 555, 670, 765, 865 nm wavelengths). The 10 years (September 1997 to September 2007) of SeaWiFS level 3 ocean colour (chlorophyll *a*) data were obtained from the GES-DISC Interactive Online Visualization ANd aNalysis Infrastructure (GIOVANNI) as part of the NASA's Goddard Earth Sciences (GES) Data and Information Services Center (DISC). While at sea daily updates of chlorophyll *a* and true colour MODIS images were received from NASA and used to assist with the selection of sample locations.

Pigment and cell count sampling and processing

On each of the 5 transects there were a series of stations in water depths of ~ 50, 100, 200, 500, 1000 and 2000 m (Fig. 1). Samples for cell counts and HPLC pigments were routinely obtained at 50, 200 and 1000 m deep stations and sometimes at the other stations. Pigment and cell samples were obtained at least every 12 hours throughout a complete tidal cycle and at the surface and deep chlorophyll *a* maximum (DCM) at the 50, 200 and 1000 m stations. Water samples were obtained from General Oceanics Niskin bottles, kept dark and cool until filtered. Samples for pigments were

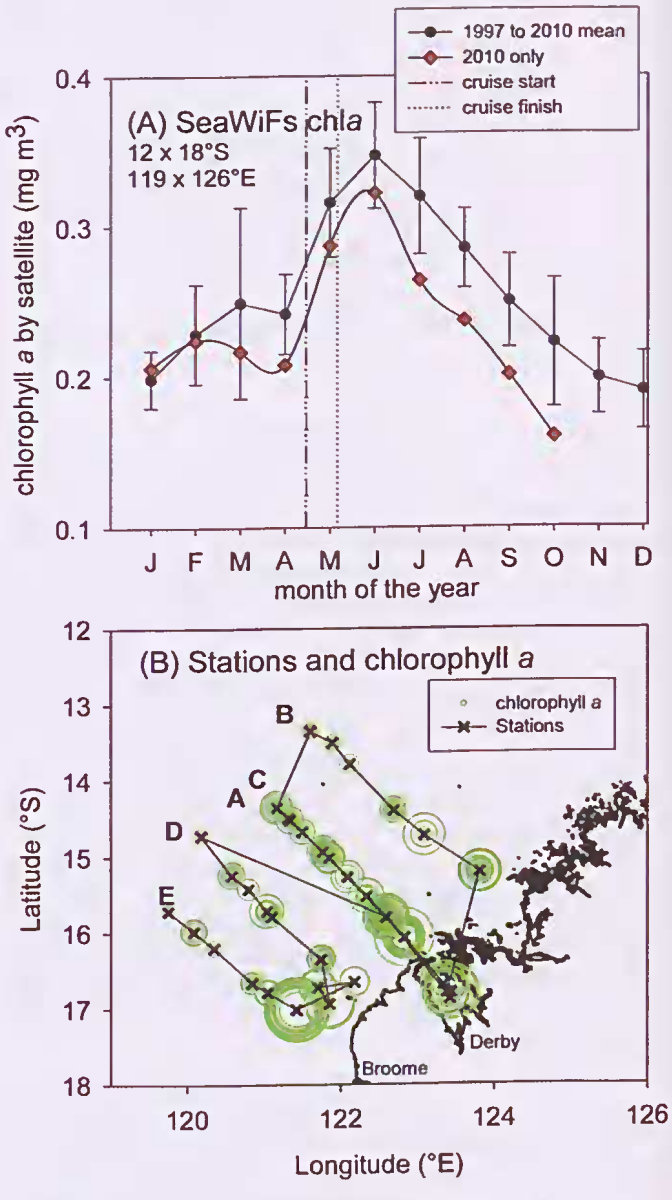

Figure 1. The phytoplankton biomass in the Kimberley region. (A) From SeaWiFS the average ± 1 standard deviation for chlorophyll *a* from 1997 to 2010 plus monthly average for 2010. (B) A map showing the stations and relative chlorophyll *a* concentrations observed during the April to May 2010 research voyage.

obtained by filtering through a range of filter types and pore sizes. Glass fibre filters (Whatman GFF®, nominally retains particles > 0.7 µm) or polycarbonate (Poretics®) filters with 0.2 µm pore sizes were considered to give 'total' chlorophyll *a*. At 105 stations size fractions were collected using a stacked filtration apparatus with a nylon mesh with 5 µm square holes followed by glass fibre filters from both the surface and chla_{max}. Other size fractions were obtained from a subset of stations (n = 44) and only the chla_{max} using Poretics® filters with 20, 10, 2 and 0.2 µm pores. Sample volumes were variable but typically ranged from 1 to 5 L. Concentrations of all pigments were determined from standards (Sigma™ or DHI Denmark). For pigment analysis by fluorometry, filtered samples were immediately extracted in 90% acetone for 24h at -20°C (Parsons *et al.* 1984) and analysed on a calibrated Turner model 10AU. Separate samples were stored in liquid nitrogen and analysed by high performance liquid chromatography (HPLC) following the methods of Van Heukelem and Thomas (2001) giving partial resolution to chlorophylls c_1 and c_2, and full resolution of MV and DV chlorophylls *a* and *b*, lutein and zeaxanthin. CHEMTAX (Mackey *et al.* 1996) was used to convert pigment concentrations into taxa. The expected ratios of various pigments to chlorophyll *a* for various taxa were derived from published literature (Andersen *et al.* 1996, Mackey *et al.* 1996; Lewitus *et al.* 2005; Latasa 2007, Marty *et al.* 2008). CHEMTAX is widely used for this purpose and is amongst the most rigorous methods but, while many phytoplankton taxa have very specific pigment signatures this is not universally true, and there is an unquantified error in the conversion from pigments to taxa. In addition to errors associated with pigments that occur in more than one taxon there are variations associated with temperature, irradiance and nutrient status (Laza-Martinez *et al.* 2007).

Water samples for phytoplankton were examined live using a Leica DM 1000 microscope capable of fluorescence excitation at 450 nm and detection at > 515 nm providing qualitative information on whether some taxa were autotrophic or heterotrophic or contained biliproteins (*e.g.* allophycocyanin, phycocyanin or phycoerythrin) or chlorophyll *a*. Samples were preserved using acid Lugol's solution (Parsons *et al.* 1984). The preserved samples were transferred to 1-litre measuring cylinders, measured and allowed to settle for at least 24 hours. After this time, approximately 90% of the volume was siphoned off and the remaining sample was transferred to a 100 ml measuring cylinder, measured and again allowed to settle. After at least 24 hours approximately 90% of the volume was siphoned off and the final volume recorded before the remaining sample was thoroughly mixed and an aliquot was taken for counting. The aliquot was put into a 1 mL Sedgwick Rafter counting chamber and examined using an inverted Olympus IX 71 microscope. For most microplankton (cells generally larger than 20 µm diameter) at least 10% of a single slide was enumerated at 100 x magnification (except for species present at very high densities which were estimated from 2%). For nanoplankton, (2 to 20 µm in diameter) the chamber was examined at 400 x magnification until at least 200 cells of the dominant nanoplankton "taxon" had been counted. Small flagellates (< 10 µm in diameter) in the nanoplankton were assigned to a single group (reported as "small flagellates") unless they could be readily identified as either dinoflagellates, chrysophytes, cryptophytes, prasinophytes or haptopytes. The use of light microscopy and relatively low magnification was insufficient to identify all taxa to species level.

Results

Based on ocean colour data the normal seasonal phytoplankton dynamics for the region of the cruise (12 to 18°S by 119 to 126°E) shows a pronounced minimum in December and a peak in June (Fig. 1A). During April 2010 the phytoplankton in this region were undergoing a strong growth phase with a > 50% increase in biomass between March and May. The cruise dates from April 14 2010 to May 5 2010 were precisely in the middle of this annual bloom (Fig. 1A). The vertical distribution of phytoplankton were assessed from the CTD casts at the 50, 200 and 1000 m stations that averaged: 58 ± 8.8 m (50 m stations), 200 m stations = 188 ± 15 m and 1000 m stations = 1045 ± 21 m (mean ± SE). The DCM ranged from 7 to 85 m; tended to be shallow and weak near shore but stronger and deeper offshore. The concentration of chlorophyll *a* declined ($P < 0.001$) with water depth (and distance from shore) from 0.44 ± 0.035 µg L^{-1} at 50 m to 0.15 ± 0.037 µg L^{-1} at 1000 m. In addition to a very strong onshore to offshore gradient in chlorophyll *a* concentrations the spatial patterns suggested increases in the vicinity of the Lacepede Islands and in the waters associated with tidal exchange in and out of King Sound (Fig. 1B).

The principal biomarker pigments (after Jeffrey *et al.* 1997) were divinyl chlorophyll *a* (=DVchl*a* found in *Prochlorococcus*), zeaxanthin (found in several taxa but largely in *Synechococcus*), 19'-hexanoyloxyfucoxanthin (=19hex found in haptophytes especially coccolithophorids), fucoxanthin (found in several taxa but largely in diatoms) and 19'-butanoyloxyfucoxanthin (=19but found largely in pelagophytes). The absolute concentrations of these biomarkers except zeaxanthin and 19hex showed very strong ($P < 0.001$) onshore – offshore gradients (Fig. 2A). The concentrations of 19but and DVchl*a* rose most strongly, ~ one order magnitude when moving offshore between water depths of 50 and 200 m. Simultaneously the concentrations of fucoxanthin fell six times from 0.14 µg L^{-1} inshore at 50 m water depth to 0.025 µg L^{-1} at 200 m water depth at the shelf break, respectively. The pigment 19hex was about two times more ($P < 0.001$) abundant at the shelf break and averaged 3 times ($P < 0.001$) more abundant at the chla_{max} than at the surface at the shelf break or offshore stations (data not shown).

The biomarker pigments normalized to chlorophyll *a* show taxonomic trends that can be distinguished from trends due to variation in biomass. Off the Kimberley coast the ratios of all 5 major biomarker pigments normalized to chlorophyll *a* showed considerable change ($P < 0.001$) with water depth (Fig. 2B). The ratios of zeaxanthin, 19hex, 19but and DVchl*a* to chlorophyll *a* all rose with increasing water depth while fucoxanthin:chl*a* fell. A number of other biomarker pigment:chlorophyll *a* ratios were maximal in the chla_{max} found at the shelf break. These included peridinin (=photosynthetic dinoflagellates), prasinoxanthin, neoxanthin,

Table 1

Phytoplankton identified from the Kimberley region during April and May 2010 along transects A and C (see Fig. 1 for details).

Taxa	Biovolume		Abundance	
	Mean (n=12) mL L^{-1}	Standard error mL L^{-1}	Mean Cells L^{-1}	Standard error Cells L^{-1}
Diatoms				
Amphora sp	1.98×10^{-5}	4.18×10^{-6}	5	1
Bacteriastrum spp	1.32×10^{-3}	1.40×10^{-4}	215	23
Chaetoceros spp < 10µm	6.18×10^{-4}	7.76×10^{-5}	1639	206
Chaetoceros spp > 10µm	8.48×10^{-3}	1.33×10^{-3}	1461	227
Climacodium sp	6.00×10^{-4}	1.73×10^{-4}	14	4
Cocconeis spp	6.75×10^{-5}	1.95×10^{-5}	12	4
Coscinodiscus spp	1.02×10^{-3}	2.34×10^{-4}	5	1
Cylindrotheca (=Nitzschia) closterium	7.45×10^{-4}	5.32×10^{-5}	1380	98
Dactyliosolen spp	6.59×10^{-3}	1.19×10^{-3}	262	48
Diploneis sp	1.41×10^{-5}	4.06×10^{-6}	4	1
Fragilariopsis spp	6.60×10^{-6}	1.90×10^{-6}	19	5
Gossleriella tropica	7.08×10^{-4}	1.51×10^{-4}	5	1
Grammatophora sp	2.97×10^{-5}	8.57×10^{-6}	7	2
Guinardia spp	1.39×10^{-2}	3.69×10^{-3}	209	47
Hemiaulus spp	9.57×10^{-4}	1.29×10^{-4}	66	9
Leptocylindrus spp	2.19×10^{-3}	1.29×10^{-4}	740	79
Lithodesmium sp	5.03×10^{-3}	1.07×10^{-3}	5	1
Navicula spp	2.91×10^{-3}	5.02×10^{-4}	273	19
Planktoniella sol	6.67×10^{-4}	1.93×10^{-4}	8	2
Pleurosigma spp	2.45×10^{-5}	7.08×10^{-6}	4	1
Porosira sp	5.54×10^{-3}	1.57×10^{-3}	31	6
Proboscia spp	2.24×10^{-2}	3.45×10^{-3}	20	3
Pseudo-nitzschia spp	7.89×10^{-4}	7.94×10^{-5}	3875	393
Rhizosolenia spp	9.66×10^{-3}	1.03×10^{-3}	176	20
Skeletonema spp	3.30×10^{-4}	6.71×10^{-5}	200	41
Synedra sp	9.81×10^{-4}	1.68×10^{-4}	60	13
Thalassionema spp	6.06×10^{-4}	7.65×10^{-5}	152	22
Thalassiosira spp	2.48×10^{-4}	3.17×10^{-5}	56	7
Trigonium spp	1.24×10^{-3}	3.59×10^{-4}	2	1
Total diatoms	8.77×10^{-2}	7.69×10^{-3}	10908	804
Dinoflagellates				
Small dinoflagellates 6–8µm	2.77×10^{-3}	1.55×10^{-4}	9175	515
Amphidinium spp	7.69×10^{-6}	1.03×10^{-6}	34	5
Ceratium spp	1.10×10^{-3}	1.74×10^{-4}	18	3
Cochlodinium sp	8.34×10^{-5}	2.41×10^{-5}	7	2
Dinophysis spp	6.52×10^{-5}	1.88×10^{-5}	4	1
Gymnodinium spp	6.21×10^{-5}	5.38×10^{-6}	84	7
Gyrodinium spp	1.62×10^{-2}	1.47×10^{-3}	156	14
Ornithocercus sp	2.62×10^{-5}	7.58×10^{-6}	4	1
Oxytoxum sp	6.26×10^{-4}	8.74×10^{-5}	29	4
Prorocentrum spp	4.77×10^{-5}	8.71×10^{-6}	18	3
Protoperidinium spp	1.94×10^{-4}	5.61×10^{-5}	4	1
Scrippsiella sp	1.80×10^{-4}	2.01×10^{-5}	38	4
Total dinoflagellates	2.13×10^{-2}	1.56×10^{-3}	9573	515
Flagellates				
Chrysophytes	2.39×10^{-4}	4.18×10^{-5}	304	53
Cryptophytes	8.63×10^{-3}	6.71×10^{-4}	10992	854
Small unidentified flagellates (3 to 10 µm)	7.89×10^{-2}	3.10×10^{-3}	384542	14707
Prasinophytes	7.65×10^{-4}	5.91×10^{-5}	2921	226
Haptophytes (includes coccolithophorids)	4.76×10^{-3}	1.70×10^{-4}	9090	323
Total flagellates	9.32×10^{-2}	3.53×10^{-3}	407849	15389
Cyanobacteria				
Trichodesmium	3.40×10^{-3}	5.85×10^{-4}	185	23
Other				
Mesodinium rubrum	4.06×10^{-3}	1.17×10^{-3}	192	55

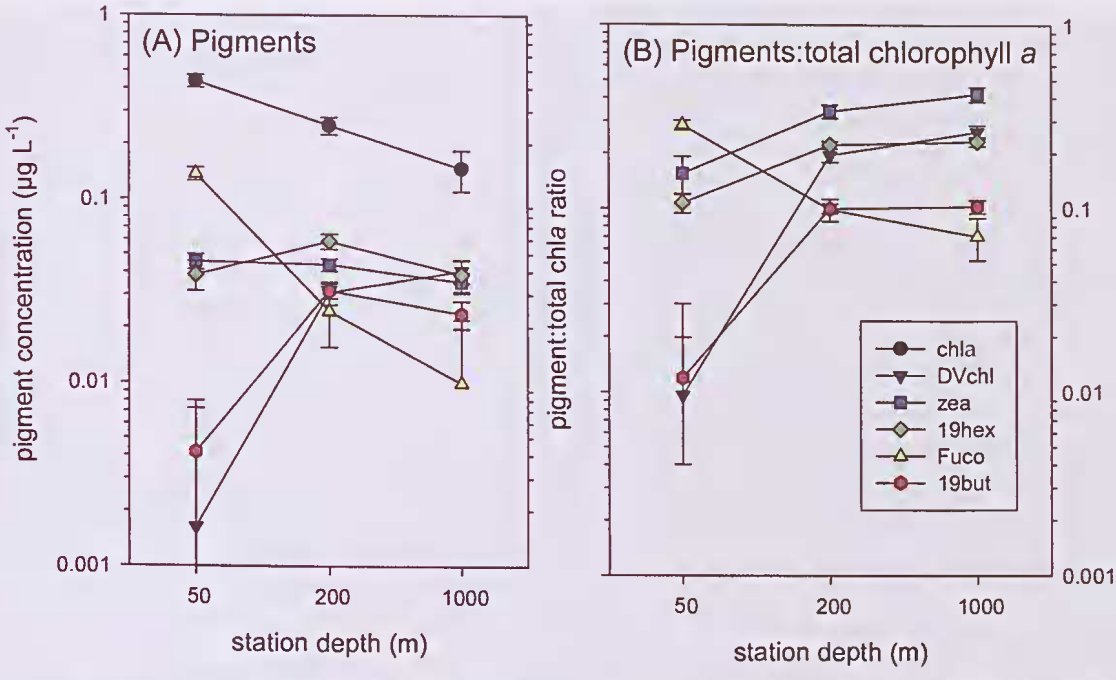

Figure 2. Trends in the concentrations of phytoplankton marker pigments with water depth. Samples were obtained from the surface (0 m) and chlorophyll *a* maximum (7 to 85 m). (A) Strong declines in absolution concentrations of zeaxanthin (*Synechococcus*) and divinyl chlorophyll *a* (*Prochlorococcus*), 19'-butanoyloxyfucoxanthin (pelagophytes), 19'-hexanoyloxyfucoxanthin (haptophytes and coccolithophorids) all increasing offshore, while fucoxanthin (diatoms) fell. (B) Trends in marker pigments normalized to chlorophyll *a* showing community composition changes.

violaxanthin (all indicators of prasinophytes or related algae) and alloxanthin. The latter is synthesized by cryptophytes. All specimens of the dinoflagellate, *Dinophysis miles*, observed live were pigmented with plastids that fluoresced strongly orange under 460 nm excitation (Fig. 3).

The ratios of most pigments to chlorophyll *a* showed significant ($P < 0.001$) trends with sample depth. The exception was the 19hex:chl*a* ratio of 0.18 at the surface and 0.20 at the chla_{max}. Ratios of fucoxanthin and 19but to chlorophyll *a* both rose with sample depth ($P < 0.001$). DVchl*a* and zeaxanthin to chlorophyll *a* fell ($P < 0.001$) with sample depth by 2.2 and 5.2 times, respectively.

The CHEMTAX results showed *Synechoccocus* was the dominant taxon in the region peaking at 55% of the biomass offshore (1000m) and in the surface waters (Fig. 4A). In contrast diatoms were ~ 10 times more abundant in the coastal zone (50 m) than further offshore. During the cruise true colour MODIS images provided evidence of patches that appeared to be relatively small coccolithophorid blooms. The species or genera of coccolithophorids were not easily identified in our routine cell counting but will be enumerated using specialized methods (Henderiks and Torner 2006). As discussed above the spatial distribution of the coccolithophorid marker pigment (19hex) was maximal at the chla_{max} found at the shelf break. Yet the ratio of 19hex:chla and the CHEMTAX output show the relative distribution of haptophytes, was not significantly different with depth ($P = 0.465$). Haptophytes were quite consistently about ~ 20% of the total biomass at the surface and at the chla_{max} in all water depths. *Prochlorococcus* was less than 1% of the biomass inshore (50m) and rising sharply to 9 to 22% of the biomass at the shelf break and further offshore. Similarly pelagophytes were found primarily offshore and strongly biased towards the bottom of the euphotic zone being 3 to 4 times more abundant in the chla_{max} than at the surface. A range of taxa including: chlorophytes, prasinophytes and euglenophytes, could have contributed some of the pigments assigned to prasinophytes in this CHEMTAX analysis. This relatively broad taxonomic group was more abundant in 50 and 200 m of water than in 1000 m.

A number of phytoplankton taxa were identified to genus or species using light microscopy. The contribution of these to the total phytoplankton biomass was determined by aggregating to genera and estimating the biovolumes (Table 1). In general the phytoplankton biomass was dominated by large diatoms and dinoflagellates (Table 1, Fig. 3). At the level of genera the large diatom *Proboscia* was the greatest component of the phytoplankton with an average biovolume of 2.2 $x10^{-2}$ mL per litre of seawater. The second greatest biomass was from the dinoflagellates where the large species in the genus *Gyrodinium* were the greatest biovolumes. Three species from the widespread genus *Guinardia* were tentatively identified (*striata, flaccida, delicatula*) and in total these represented the third largest component of the phytoplankton biomass. Large centric diatoms of the genera *Rhizosolenia* were the fourth largest component followed by large (> 10 µm) *Chaetoceros* species, *Dactyliosolen, Porosira, Lithodesmium* species. The ciliate *Mesodinium rubrum* (= *Myrionecta rubra*), a voracious consumer of cryptophytes was 9th followed by the trichome forming cyanobacterium *Trichodesmium*.

The spatial distributions of some genera were reasonably uniform while others were very patchy. Of the taxa with relatively high biovolumes in the region

Figure 3. Light micrographs of the dominant taxa found in the Kimberley region. Row 1 (L to R): *Proboscia* sp. *Gyrodinium* sp., *Guinardia* sp., *Rhizosolenia* sp.,
Row 2 (L to R) large *Chaetoceros* sp., *Dactyliosolen* sp, *Lithodesmium* sp., (~ 100 μm diameter), *Trichodesmium* sp.
Row 3 (L to R) *Navicula* sp., *Leptocylindrus* sp., *Bacteriastrum* sp., *Trigonium* sp.
Row 4 (L to R) various *Ceratium* spp., *Coscinodiscus* sp., *Dinophysis miles, Dinophysis miles* (under epifluorescence).

Mesodinium, Trigonium and *Plauktoniella* all had coefficients of variation greater than 28%, basically occurring in 1 of 12 samples analysed. In the rare samples where they occurred they tended to dominate the biomass. For example taxa found only once included: *Mesodinium rubrum* which was 14% of total phytoplankton biovolume at the chla_{max} (75 m) offshore in a 1000 m of water. *Planktoniella* was also found at 75 m in the chla_{max} but only at the shelf break (~200 m water depth) and *Trigonium* was 9% of total phytoplankton biovolume at the surface and again only at the shelf break. Genera that were high in biovolumes and relatively uniformly distributed included *Gyrodinium, Navicula* and *Nitzschia;* all with coefficients of variation ≤ 9%. The genus dominant by biovolume, *Proboscia,* was not found in any of the samples from near shore (~50 m) but in 4 of 8 samples from deeper waters.

The size distribution of phytoplankton derived from size fractionated chlorophyll *a* (please note that this includes only photosynthetic species) showed very strong ($P < 0.001$) onshore to offshore gradients (Fig. 5). The picoplankton (< 2 μm cells) were dominant everywhere but they reached their lowest fraction of the total biomass at the 50 m stations; where they were only 54% of total chlorophyll *a*. Phytoplankton cells larger than 20 μm reached 26% of the total chlorophyll *a* at these inshore stations. The portion of phytoplankton greater than 20 μm fell to 3% of total chlorophyll *a* in 1000 m of water. At the same time the proportion of picoplankton rose reaching 84 to 80% of total chlorophyll *a* in 200 and 1000 m of water, respectively.

Discussion

Cell size is one of the fundamental characteristics of a phytoplankton species and of the community because of the strong associations between cell size and the physiological traits such as nutrient and light utilization plus grazer resistance (Banse 1976, Grover 1989, Sterner 1989, Litchman *et al.* 2007). Previously the nanoplankton (>2 and <15 μm) were proposed to be the dominant phytoplankton group (Hallegraeff and Jeffrey 1984) in the Kimberley but these new results show the majority of the autotrophic phytoplankton biomass were

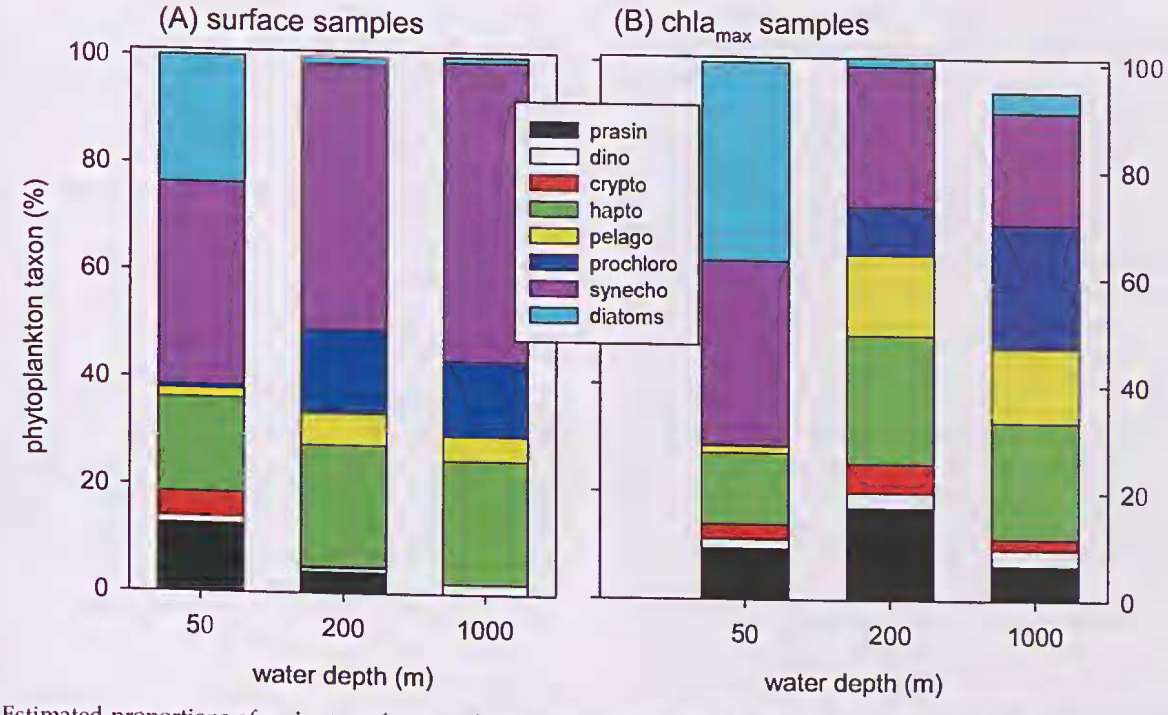

Figure 4. Estimated proportions of major taxa from marker pigments using CHEMTAX (Mackey *et al.* 1996). Abbreviations as follows: prasin = prasinophytes, dino = dinoflagellates, crypto = cryptophytes (also contains *Mesodinium rubrum* and *Dinophysis miles* (see text), hapto = haptophytes (includes coccolithophorids), pelago = pelagophytes, prochlo = *Prochlorococcus*, synecho = *Synechococcus*.

picoplankters (< 2 μm), predominantly *Synechococcus* and *Prochlorococcus*. The sampling of size fractionated chlorophyll *a* showed that the portion of autotrophic phytoplankton that were picoplankton peaked at ~ 80% in the offshore region. These very small cells with a high surface area to volume ratio and low self shading are considered well adapted to stable low nutrient and low light conditions (Raven 1987, 1998). Their very small size also confers considerable resistance to grazing by mesozooplankton and some reduction in grazing by microzooplankton (Monger & Landry 1991). A few genera, such as *Trichodesmium Proboscia, Rhizosolenia,* may also escape significant grazing pressure by virtue of their large size.

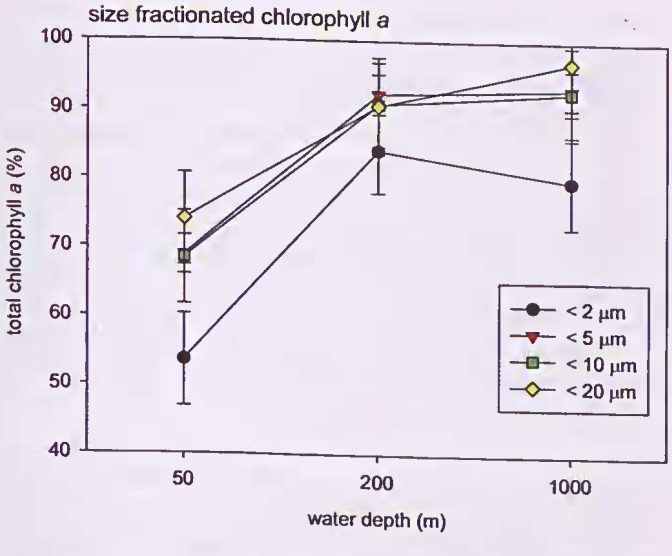

Figure 5. The proportions of chlorophyll *a* found in cells that were less than a particular size (2, 5, 10 and 20 μm) from near shore (50 m water depth) to offshore (1000 m water depth). For example, 54% of chlorophyll *a* was found in cells less than 2 μm where the bottom was 50 m below the surface.

The sampling regime implemented in 2010 provided strong evidence of onshore to offshore gradients in biomass, community composition, depth distributions and size structure in the Kimberley region. In the offshore samples the vertical differences in phytoplankton biomass and community composition were much greater between the surface and the chla_{max} than those observed near shore. Offshore the deep chla_{max} had ~ 100% more pelagophytes than the surface. The pelagophytes are a picoeukaryote taxon found broadly across the Indian Ocean (Not *et al.* 2008) and along most of the west Australian coast they increase their dominance with depth (Thompson *et al.* 2010). The presence of a deep chlorophyll *a* maximum and significant community differentiation between the surface and the chla_{max} indicates the stability of the water column in these locations must have exceeded many generations of the phytoplankton (Cullen & Lewis 1988, Cullen 1990) preceding the cruise in April 2010.

The phytoplankton taxa collected during the April to May 2010 survey (herein) and observed under the light microscope were broadly consistent with those reported from the general region (Conkright *et al.* 2002) and from Northwest Shelf to the Gulf of Carpentaria by Wood (1964) and Hallegraeff & Jeffrey (1984). Of the microplankton, the diatom genera *Proboscia* (formerly *Rhizosolenia alata*), *Rhizosolenia, Chaetoceros, Bacteriastrum, Navicula,* and the dinoflagellates *Gyrodinium, Ceratium* and the cyanobacterium *Trichodesmium* were all relatively abundant in the region. The three species of *Guinardia* found in 2010 were reported as common or rare under previous names *Guinardia delicatula* (formerly *Rhizosolenia delicatula*), *Guinardia flaccida* (formerly *Rhizosolenia*

flaccida), *Guinardia striata* (formerly *Rhizosolenia stolterfothii*) by Hallegraeff & Jeffrey (1984). Reported as rare (Hallegraeff & Jeffrey 1984), or not previously reported from the region, were some genera found to be abundant in 2010 including: *Dactyliosolen, Porosira, Lithodesmium, Leptocylindrus, Trigonium* and *Coscinodiscus*. Species of *Leptocylindrus, Coscinodiscus* and *Trigonium* were reported as rare by Hallegraeff & Jeffrey (1984) and they were infrequently observed in 2010 but their large size meant that they were in the top 15 genera by biovolumes. *Dactyliosolen fragilissimus* (formerly *Rhizosolenia fragilissima*) is cosmopolitan (Tomas 1996) but possibly a new record for the region (Conkright *et al.* 2002). Species from the genus *Porosira* are mostly temperate to polar in distribution (Conkright *et al.* 2002) but have been previously reported from the Indian Ocean (Tomas, 1996). Finally *Lithodesmium* has been recorded once from the Tasman Sea (Conkright *et al.* 2002), widely from the Philippine Sea Bantu Sea and other warm water regions (Tomas, 1996). It is not clear whether the apparent changes in abundance of these genera represent significant changes in the phytoplankton community within this region or have some other cause(s). Based upon the limited spatial and temporal sampling conducted to date we can only hypothesize based on principles why the abundance of some diatom species in the Kimberley region were much greater relative to samples from Northwest Shelf to the Gulf of Carpentaria (*e.g.* Hallegraeff & Jeffrey 1984).

The phytoplankton community near shore in the Kimberley region was much more abundant than offshore. With an average of ~ 0.5 µg chlorophyll *a* L^{-1} it also appears more abundant than most similar regions along the west coast of Australia (Thompson *et al.* 2010) although this may reflect the limited temporal sampling. Relative to offshore it also had a significantly greater component of larger cells and these were mostly diatoms (as above). The diatoms observed in the near shore region tended to be relatively evenly distributed throughout the water column and strongly pigmented. Diatoms with a relatively homogenous vertical distribution and high in pigmentation suggest a well mixed water column with fluctuating but low average irradiance (Litchman & Klausmeier 2001). The medium size of many of these near shore diatoms could be expected to make them easy prey for many zooplankton species (Irigoien *et al.* 2000) and they may rely on rapid growth (r-selected) to escape this predation pressure (Litchman *et al.* 2007). The nutrients to support this substantial near shore phytoplankton community may be advected on to the shelf from the strong tidal exchanges (Tranter & Leech 1987), mixed vertically due to breaking internal waves or some other mechanism yet to be elucidated.

From the MODIS (Moderate Resolution Imaging Spectroradiometer) satellite images there were small blooms of coccolithophorids (haptophytes) present during the 2010 cruise. A number of coccolith bearing species have been previously reported from the region although their abundance was only quantified as between 1 and 100% of the total phytoplankton biomass (Hallegraeff & Jeffrey 1984). Based on pigments during the 2010 research voyage coccolithophorids were approximately 20% of the total phytoplankton biomass, most abundant at the shelf break and they were relatively deep in the euphotic zone. The coccolithophorid blooms that are frequently reported across this region from the Kimberley to Cape York (*e.g.* Brown & Yoder 1994) are important on a global scale yet we know almost nothing about the ecology of these species in this region or the impacts of these blooms. Given the role these taxa play in the global carbon cycle this is a serious gap in our understanding of the carbon budget and potential acidification of the Australian environment. Samples from 2010 are being examined at greater magnification (to identify and enumerate the coccolithophorids).

Cryptophytes are rarely observed in phytoplankton samples yet their marker pigment, alloxanthin, is often found (Schulter & Mohlenberg 2003). In the Kimberley region cryptophytes were common in the phytoplankton relative to other samples from west Australia (*e.g.* Thompson *et al.* 2010). The mixotrophic ciliate, *Mesodinium rubrum*, which contains plastids derived from cryptophytes (Taylor & Blackbourn 1970) was spatially heterogeneous but abundant overall. *M. rubrum* has an exceptional swimming speed of ~ 7 mm S^{-1} (Lindholm 1985) or an order of magnitude faster than most dinoflagellates. This speed gives it a unique capacity to move vertically reducing predation (Jonsson & Tisleius 1990), to utilize ephemeral nutrient patches and maximize irradiance. The dinoflagellate *Dinophysis miles* was also patchy in its distribution and its orange, rather than red, fluorescence indicated biliprotein pigments. It would appear that *D. miles*, like its better studied congeners *D. acuminata, D. caudate, D. fortii, D. infundibulus*, is consuming *M. rubrum* (Parke *et al.* 2010). The orange fluorescing plastids in *D. miles* are proposed to be obtained from *M. rubrum* but originally derived from cryptophytes and to contain both biliproteins and alloxanthin. This mode of mixotrophy provides some additional niche diversity and seems to be more common feature of phytoplankton found in the relatively stability of tropical ecosystems (Troost *et al.* 2005a, Troost *et al.* 2005b).

The phytoplankton of this region are likely to be limited by the availability of nitrogen (Elser *et al.* 2007). *Trichodesmium* is the source of 50% the nitrogen used by phytoplankton in the global ocean (Mahaffey *et al.* 2005). *Trichodesmium* is an important component of the phytoplankton of the Kimberley region and is likely to be an important nitrogen source supporting the regional ecology (*e.g.* Furnas *et al.* 1995, Capone *et al.* 1997). In addition the effects of rising CO_2 are predicted to double the growth rate, carbon fixation and N_2 fixation by *Trichodesmium* (Hutchins *et al.* 2007; Levitan *et al.* 2007). Thus *Trichodesmium* is likely to be even more important in the future acidified ocean and there is significant need for more attention to be focused on this pathway of N input to the Australian ecosystem.

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